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SIMULATION OF RESPIRATION IN MICROBIAL SLIME FILMS

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Clemson University

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SIMULATION OF RESPIRATION IN MICROBIAL SLIME FILMS

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27 Abstract
A matrix method has been developed for calculating dissolved oxygen profiles near and within slimes of microorganisms bathed by flowing nutrient medium. The matrix manipulations and recursions necessitated by non-linearities in the equations are carried out in FORTRAN programming on an IBM-360 computer. The computer provides numerical output and plots of dissolved oxygen concentration versus either distance from the slime surface or distance along the slime. A diffusion mechanism is assumed, and equations are derived from mass balances for oxygen and from the laws of diffusion. Although an exact mathematical model required partial differential equations, an excellent approximation is obtained by using ordinary differential equations to describe exchanges between discrete sections of the nutrient medium and between discrete elements of the slime. Two situations which were studied in detail were the development of steady-state oxygen profiles as medium flowed past a microbial slime and the profile established at an infinite distance along a microbial slime.

The computer models can be used to study oxygen transfer and are particularly valuable in performing sensitivity tests on various parameters. There is confirmation of the validity of the model from its ability to approximate experimentally determined oxygen profiles when using oxygen diffusivities and uptake rates derived from tests with living systems. The model is ready for further validation and subsequent use as an adjunct to experiments with real systems. (Bungay-Clemson) (ESRI)

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ABSTRACT

A matrix method has been developed for calculating dissolved oxygen profiles near and within slimes of microorganisms bathed by flowing nutrient medium. The matrix manipulations and recursions necessitated by non-linearities in the equations are carried out in FORTRAN programming on an IBM-360 computer. The computer provices numerical output and plots of dissolved oxygen concentration versus either distance from the slime surface or distance along the slime. A diffusion mechanism is assumed, and equations are derived from mass balances for oxygen and from the laws of diffusion. Although an exact mathematical model required partial differential equations, an excellent approximation is obtained by using ordinary differential equations to describe exchanges between discrete sections of the nutrient medium and between discrete elements of the slime. Two situations which were studied in detail were the development of steady-state oxygen profiles as medium flowed past a microbial slime and the profile established at an infinite distance along a microbial slime.

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CONTENTS

Section		Page
I	Conclusions	1
II	Recommendations	3
III	Introduction	5
IV	Development of the Model	7
V	Results	13
VI	Supporting Studies	27
VII	Discussion	31
VIII	Acknowledgement	33
IX	References	35
X	Symbols	37
XI	Patents and Publications	39

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FIGURES

		PAGE
1	Sketch of Slime and Medium	8
2	Flow Chart for Computer Program	11
3	Effect of Distance Along Slime on Dissolved Oxygen Profile	14
4	Effect of Depth of Medium on Oxygen Concentration	15
5	Effect of Slime Thickness on Dissolved Oxygen Profile	16
6	Effect of Inlet Oxygen Concentration on Dissolved Oxygen Profile	17
7	Effect of Oxygen Diffusivity in the Liquid on Dissolved Oxygen Profile	18
8	Effect of Interface Resistance on Dissolved Oxygen Profile	19
9	Effect of Oxygen Diffusivity in the Slime on Dissolved Oxygen Profile	20
10	Effect of Value Selected for Maximum Uptake Rate on Dissolved Oxygen Profile	21
11	Effect of Constant in Denominator of Monod Equation on Dissolved Oxygen Profile	22
12	Effect of Flow Velocity of Medium on Dissolved Oxygen Concentration in the Deepest Slime Layer for Various Slime Thicknessess	23

SECTION I

CONCLUSIONS

1. A computer model is available which gives oxygen concentrations near and within a respiring microbial slime. It can be used to estimate parameter sensitivity and to aid research with living systems.

2. Each parameter of the model has been tested over a range of values to determine its effects and its importance relevant to other parameters.

3. The parameters with the most significant effects on the shapes of oxygen profiles were oxygen diffusivity in the nutrient medium, oxygen diffusivity in the slime, and interface resistance to oxygen transfer.

4. Major improvements in the removal of wastes from solutions flowing past microbial slimes are not likely to come from changes in hydraulic design or from varying the slime thickness. Resistances to mass transfer are inherent properties of the slime; significant increases in rates could arise from increasing diffusivity by developing a less dense slime.

SECTION II

RECOMMENDATIONS

This effort on modeling oxygen transfer seems to have been quite successful, thus there is little justification for attempting to improve it. However, the present model has had only limited verification, and deficiencies may be found.

Further modeling efforts should concentrate on extending the existing model to consider additional effects such as temperature and transient changes in parameters. Substrate diffusion should be modeled because only oxygen-limited cases have been studied so far. In many natural waters, concentrations of organic matter are relatively low, and respiration rates are controlled by substrate limitations.

Experimental measurements of oxygen profiles and of microbial ecology in slime films should be performed and the results fitted into the computer studies. Continued interplay of laboratory investigations and computer simulations have great promise for gaining greater understanding of the role of microbial slimes in natural waters and in waste treatment systems.

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SECTION III

INTRODUCTION

Microorganisms growing in slime layers attached to solid surfaces can play major roles in bodies of water, and assimilation of organic substances by slime organisms is the basis for waste treatment by trickling filters. Engineers are interested in wall growth in reservoirs, the benthic organism contribution to metabolic activities in lakes and streams, slimes which form in pipes and conduits, and soil characteristics affected by growth in the "voids." Organisms are important in solids which are percolated by waste waters as in septic tank drainage fields.

To optimize processes based on microbial slimes, it is essential to have better understanding of the mechanisms for mass transfer of oxygen, carbon dioxide, and nutrients. The effects of temperature, hydraulic regime, nutrient concentration, ionic environment, and shifts in the microbial population require study. Previous investigators of the metabolic activities of microbial slimes have estimated nutrient uptake and oxygen uptake by the film by measuring changes in the medium. Only gross averages of film characteristics can be obtained by such approaches. The development of a microelectrode for dissolved oxygen now makes it possible an investigation on a cellular level of oxygen transfer and utilization. Our research has shown that the diffusivity of oxygen and the respiration rate can be calculated at specific locations within the film.

The objectives of the project were to develop a model for simulating oxygen transfer and respiration in microbial slime systems, to verify the validity of the model, and to gain new insight into the behavior of the microbial slimes by computing the expected effects of various parameters on dissolved oxygen profiles. The model is intended as an aid to research so that ideas gained from modeling can be tested in real systems and conversely new data can be used to refine features of the model. There were plans to model not only oxygen concentration but nutrient concentration, carbon dioxide evolution, and growth of the microorganisms. This proved to be too ambitious for a one-year project, but the College of Engineering at Clemson University has decided to support continuing efforts to improve the model and to add new features. The funds will come from a large N.S.F. grant for real-time and hybrid computation.

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SECTION IV

DEVELOPMENT OF THE MODEL

The situation to be simulated consisted of a stationary slime layer of uniform thickness growing on a flat supporting surface with medium flowing past. A schematic view of the physical layout is shown in Figure 1. The liquid medium passing over the microorganisms contained dissolved oxygen and nutrients. The number of suspended microorganisms in the liquid was very small and thus they were neglected. The liquid was assumed to have a constant density and diffusivity throughout. Within the slime the density and diffusivity were also regarded as constant. The flow of the liquid past the slime layer was at low enough velocity to be laminar. Mass transfer of oxygen from the liquid medium to the slime layer was considered to be by molecular diffusion. In a turbulent flow, eddy currents become the predominant mechanism of mass transfer. The assumption, in addition, was made that the system was at steady state. That is, the conditions at any fixed point in the system did not change with time. For variation of distance in the liquid phase of the model, dissolved oxygen changes were important only in the layers close to the slime; thus, the upper layers of the liquid film were assumed to be at the same dissolved oxygen concentration as the feed stream.

Figure 1 indicates that x and y space coordinates; δ, the liquid film thickness; and L, the biological film thickness. Description of the velocity profile $u_y(s)$ for the liquid film was represented by Equation (1),

$$u_y(x) = u_{max} [1 - (x^*/\delta)^2] \qquad (1)$$

where u_{max} is the maximum liquid flow velocity at the liquid surface, and $x^* = \delta - x$, where x is the distance above the slime surface into the liquid. The liquid flow was in the y coordinate direction.

Oxygen dissolved in the liquid was transported to the respiring slime by molecular diffusion. Mass transfer by molecular diffusion is described by Fick's law (1) as

$$N = -\mathcal{D} \frac{\partial C}{\partial x} \qquad (2)$$

in which N is the molar flux of a given component per unit area per unit time. Equation (2) states that the flux N is directly proportional to the concentration difference or driving force. As the microrganisms use oxygen, a greater concentration difference arises between the liquid and slime; thus, more oxygen diffuses from the liquid into the oxygen respiring regions of the slime.

In order to formulate a mathematical model as a differential equation, imagine an infinitesimal element of dimension Δx, Δy, and W. The law of conservation of mass may be applied to this volume through which medium with dissolved oxygen is flowing. Oxygen used by suspended microorganisms is neglected. For constant density and diffusivities a general equation of continuity for the system is

$$u_x \frac{\partial C}{\partial y} + \mathcal{D} \frac{\partial^2 C}{\partial x^2} - R = \frac{\partial C}{\partial t} \qquad (3)$$

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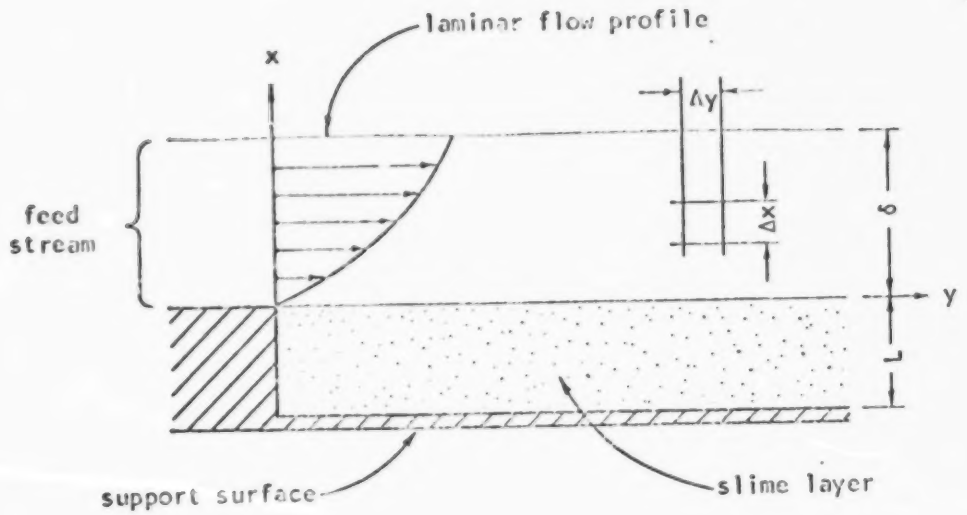

FIGURE 1

Sketch of Slime and Medium

where C is the dissolved oxygen concentration. Remember that the system is at steady state; thus, C/ t is zero. In the liquid medium there is no oxygen utilization by microorganisms; thus, R is zero. Within the slime layer, liquid flow is zero. In the liquid the basis mass balance equation is

$$u_x \frac{\partial C}{\partial y} + \mathcal{D} \frac{\partial^2 C}{\partial x^2} = 0 \qquad (4)$$

and within the slime layer

$$\frac{\partial^2 C}{\partial x^2} - R = 0 \qquad (5)$$

Solution of the partial differential equations, Equations (4) and (5), using computer methods demanded the division of the physical system into discrete slices. In Figure 1 imagine a grid defining the boundaries between many discrete rectangular volumes. Consider an element of volume within the liquid point. The upper, lower and side faces of this element are parallel to the y and x axes, respectively. Depth of this element is W, the width of the stream. Dissolved oxygen in the flowing liquid stream flows through this stationary element. Some oxygen, however, diffuses out across the lower face toward the slime; but the major portion of the dissolved oxygen flows out with the liquid across the opposite face in the y direction. In the same manner, consider an element within the slime where no liquid is flowing. Oxygen enters this volume only by diffusion from above. Occurring within the volume is the utilization of the dissolved oxygen by the slime. Within each volume block ordinary differential equations could express oxygen transfer. Groups of these discrete volumes were defined as either slices or layers. A slice is a group of volumes forming a vertical segment as blocks stacked one above the other. Lateral groups of volumes are referred to as layers. For each discrete volume of the system in Figure 1 a linear mass balance equation with constant coefficients was written. These equations were grouped according to slices. A system of finite difference equations were written using subscript notation to distinguish slices. These equations are linear with constant coefficients. Therefore, this system of equations may be written in the form in which one matrix equation replaces the entire system of scalar equations.

In matrix notation the equation is

$$\overline{A}\,\overline{C} - \overline{R} + \overline{B} = \overline{0}. \qquad (6)$$

Matrix $\overline{A}$ is the constant coefficient matrix elements which are functions of flow velocities, area, and diffusivities; $\overline{B}$ is a vector of initial conditions and flow velocities; $\overline{R}$ is a vector of oxygen utilization rates; and $\overline{C}$ is the solution of the matrix equation representing dissolved oxygen concentrations at each layer within the slide. Using matrix algebra, the solution to Equation (6) may be determined. Addition of the vector $\overline{R} - \overline{B}$ to beth sides of Equation (6) results in Equation (7),

$$\overline{A}\,\overline{C} = \overline{R} - \overline{B}. \qquad (7)$$

A square matrix when multiplied by its inverse will yield the identity matrix, $\overline{I}$. Consequently, multiplying both sides of Equation (9) by the inverse of the coefficient matrix, $\overline{A}^{-1}$. gives;

$$\overline{I}\,\overline{C} = \overline{A}^{-1}\,(\overline{R} - \overline{B}). \qquad (8)$$

The solution is

$$\overline{C} = \overline{A}^{-1}\,(\overline{R} - \overline{B}). \qquad (9)$$

This vector $\overline{C}$ is the solution to the system of equations. The elements of vector $\overline{C}$ represent dissolved oxygen concentrations for a slice. An oxygen profile is described when the concentrations from vector $\overline{C}$ are plotted as a function of depth relative to the slime surface. Progression downstream with the model was achieved by solving the system of mass balance equations for the next adjacent slice. When changing slices the flow of dissolved oxygen in to these slice elements comes from the previous slice. Thus, the oxygen entering the slice was the solution for the previous slice.

At an infinite distance downstream changes in oxygen concentration no longer vary with downstream distance. Hence, simulation at an infinite distance downstream may be modeled by substituting the conditions $\partial C/\partial y = 0$ into Equation (3). The same outcome results, in fact, when u_x is zero. A computer model was constructed, therefore, for an infinite distance by substituting F = 0 into the equations.

Description of Computer Program

By means of branching within the program three situations were simulated by the computer program; one, profiles were generated along the stream to a specified distance; second, an oxygen profile was simulated at an infinite distance downstream; and third, a combined simulation of both stream and infinite models were generated.

A simplified flow diagram of the oxygen transfer routine is shown in Figure 2. For the entire simulation only one matrix inversion was needed. This was because matrix $\overline{A}$ was composed of coefficients which did not change when y was changed. Consequently, an entire simulation of the stream could be achieved with one matrix inversion. Tremendous amounts of computer time were saved by having the inversion routine outside of the iteration loop.

Matrix A had the unique feature that its nonzero elements were only on the main diagonal and adjacent off diagonals. This is an example of a tridiagonal matrix. Computer methods for inverting tridiagonal matrices are much simpler than inversion techniques for matrices without the tridiagonal feature.

Within the liquid, oxygen utilization was not present. Therefore, only flow terms were incorporated into vector $(\overline{R} - \overline{B})$ in the liquid. On the other hand, within the slime the vector $(\overline{R} - \overline{B})$ included R terms but velocity terms were zero.

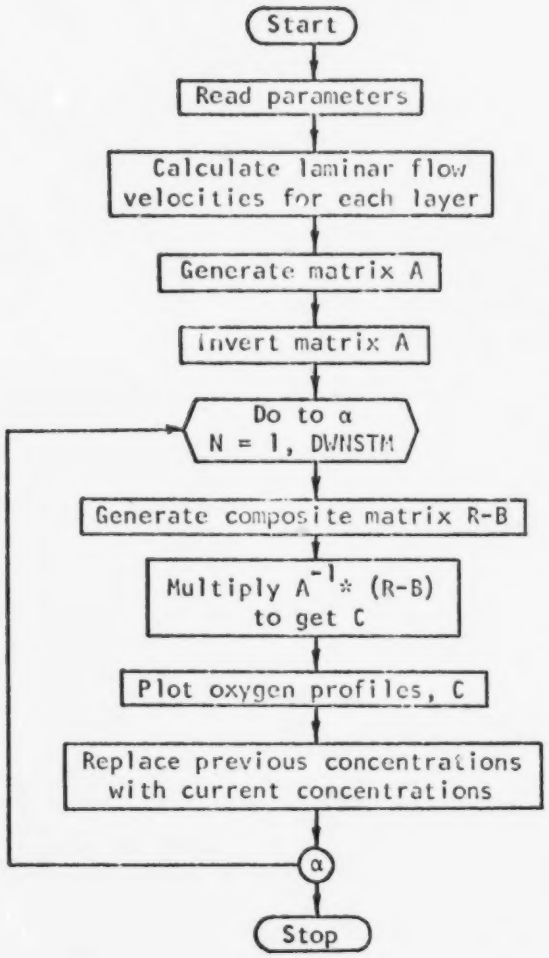

FIGURE 2

Flow Chart for Computer Program

Multiplication of the inverse of matrix $\overline{A}$ and vector $(\overline{R} - \overline{B})$ gave vector $\overline{C}$ the oxygen profile of the current slice. Certain conditions were forced upon the program in order that truncation errors in the digital computation would not affect this solution. For instance, the dissolved oxygen concentration would always in this simulation be decreasing as distances deeper into the slime were attained. Also, concentrations within a slice element would be less than or equal to the respective concentrations in a previous slice. In addition, any negative concentration was replaced with zero, since negative concentrations would imply that there existed manufacturing sorces of oxygen.

When the solution to a slice was found, progression to the next slice followed by using as initial conditions on the next slice, the solution for the current slice. These concentrations which were used as initial conditions for the next iteration were from the solution vector $\overline{C}$.

Oxygen Utilization Rate

The oxygen utilization rate, R, depends upon the oxygen concentration according to some saturation function. The relationship used in the model is

$$R = R_{max} \quad \frac{C}{C + G_L} \tag{10}$$

Matrix algebra usually requires linearity for elements of the matrix. A recursion scheme was used in which a value of R was assumed so that C could be found by matrix manipulations. This value of C was used to calculate a more accurate value of R, and the recursion was continued until R did not change more than some specified percentage.

SECTION V

RESULTS

The results of a computer run simulating oxygen profiles along the stream were plotted as shown in Figure 3 with a CALCOMP plotter subroutine supplied by the Clemson University Computer Center. The program which called upon portions of the CAPCOMP routine to plot the oxygen profile was written by Dr. D. M. Cline. Dissolved oxygen profiles are shown in 1, 10, 100, 1000, 5000 centimeters and an infinite distance downstream from the beginning of the slime. Most of the parameters which yielded these profiles were based on microprobe data (1). The estimate for maximum rate of oxygen utilization was 1.0×10^{-2} milligrams per liter per second. The greatest transition in the profile shape occurred at around 100 centimeters. Past 1000 centimeters the profile was essentially the same as the profile at infinity.

Parameter Study

Each parameter was varied in a series of computer runs to determine its affect upon the system. The oxygen profile at an infinite distance downstream was used as a parameter study model because the simulation was rapid and required only one iteration. The setting of the parameters were again based on microprobe studies (1). The value of maximum rate of oxygen consumption was selected such that the oxygen profile would stabilize in the lower slime layers at an oxygen concentration mid-range on the scale. Therefore, the effects of the other parameters were easy to discern for deviations from this intermediate profile.

Results from the parameter studies are graphed in Figures 4 through 11. The scale representing depth is distorted because the layer depth in the model was different in the liquid from that in the slime. Figure 12 shows the effect of surface flow velocity of the liquid on oxygen in the lower slime layers.

The effect of changing the depth of liquid modeled (Figure 4) indicated a deviation in the slope of the profile in the liquid region. The fact that the oxygen profile was linear to the liquid region not modeled was a result of the inherent nature of the infinite model. The source of oxygen at this infinite distance was the layer above the modeled region; the liquid only acted as a medium through which oxygen was transferred from this layer to the slime. Remember that the initial assumption that the oxygen concentration above the liquid depth modeled was the feed stream concentration. Forcing the solution to be at an infinite distance downstream imposed considerable stress upon this assumption. However, for models at reasonable distance as below 100 centimeters in Figure 3, oxygen usually will not deplete to the unmodeled region; thus, the assumption is valid.

The effect of increasing the thickness of the slime layer (Figure 5) was to lower oxygen tension in the slime. Since each slime layer received its oxygen by diffusion from the layer above, the oxygen available to the microorganisms deep within the slime was depleted by the organisms in the

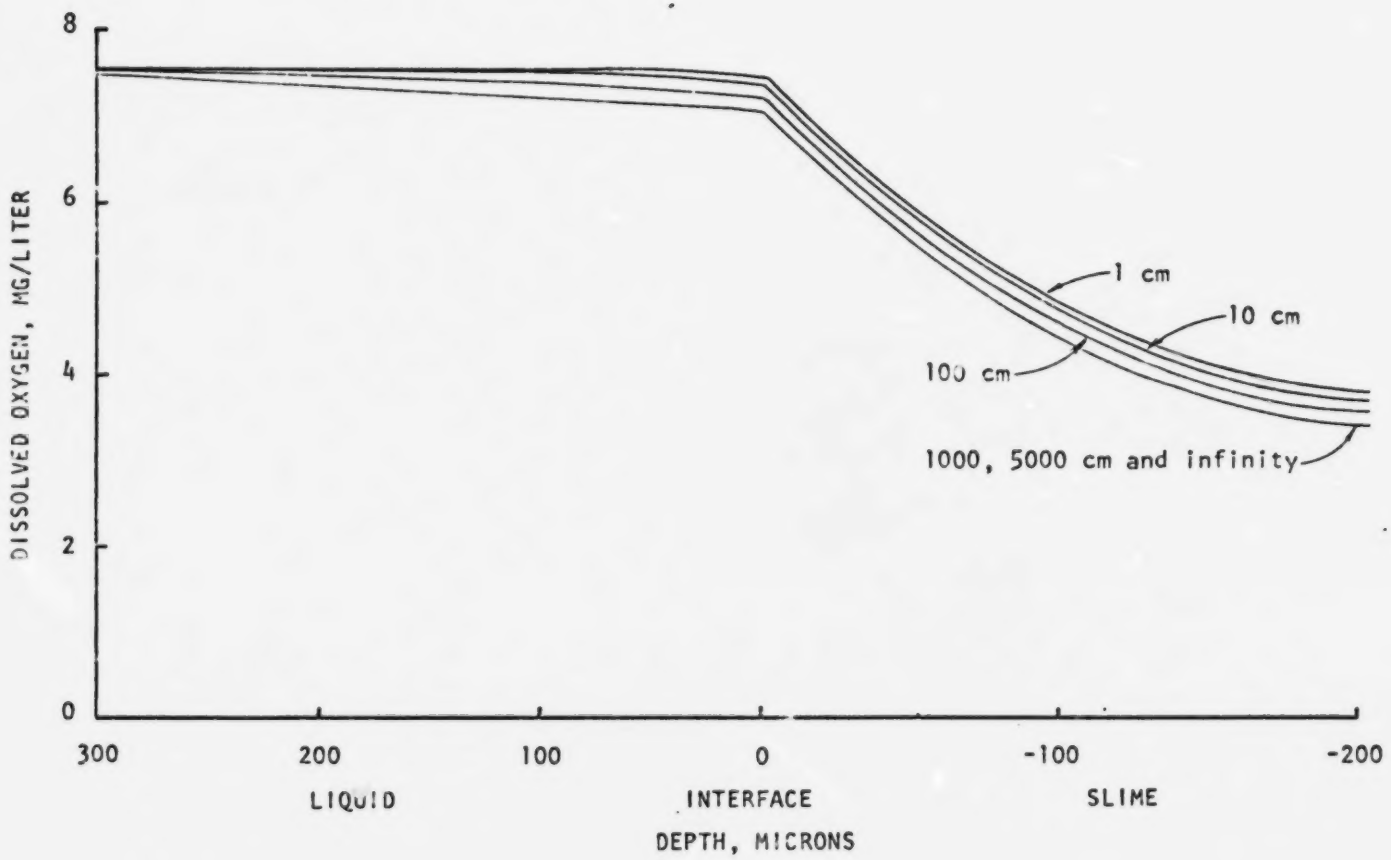

FIGURE 3

Effect of Distance Along Slime on Dissolved Oxygen Profile

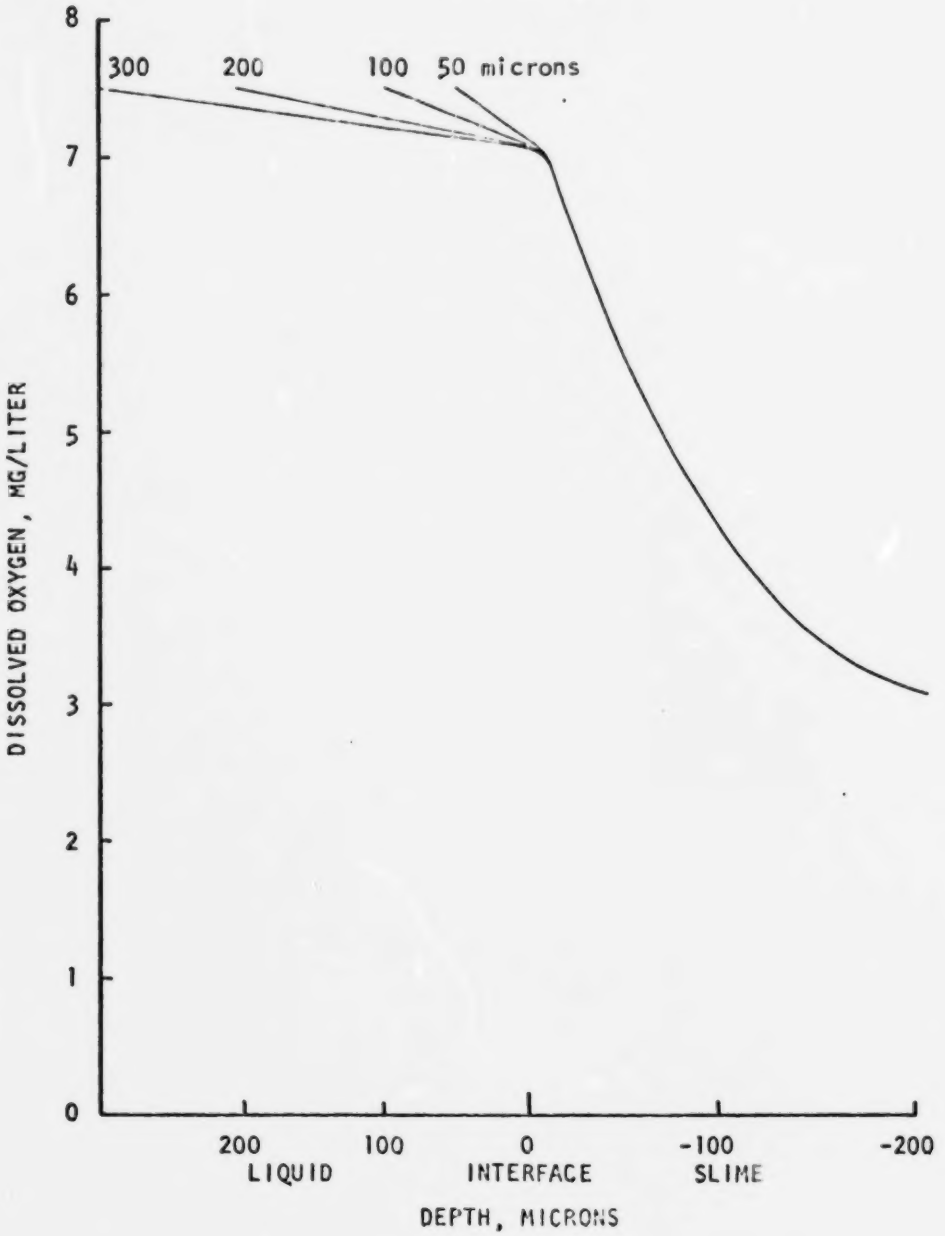

FIGURE 4

Effect of Depth of Medium on Oxygen Concentration

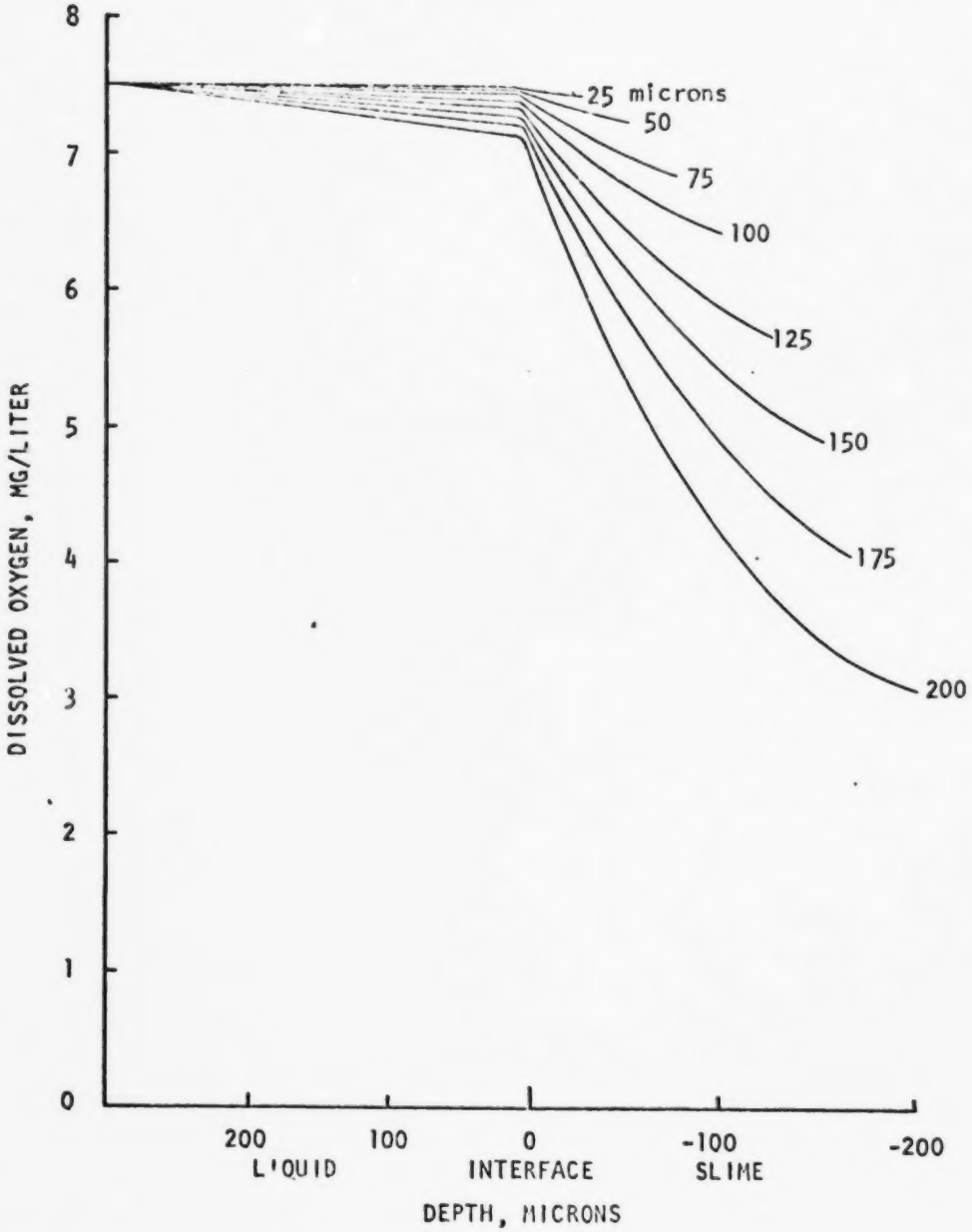

FIGURE 5

Effect of Slime Thickness on Dissolved Oxygen Profile

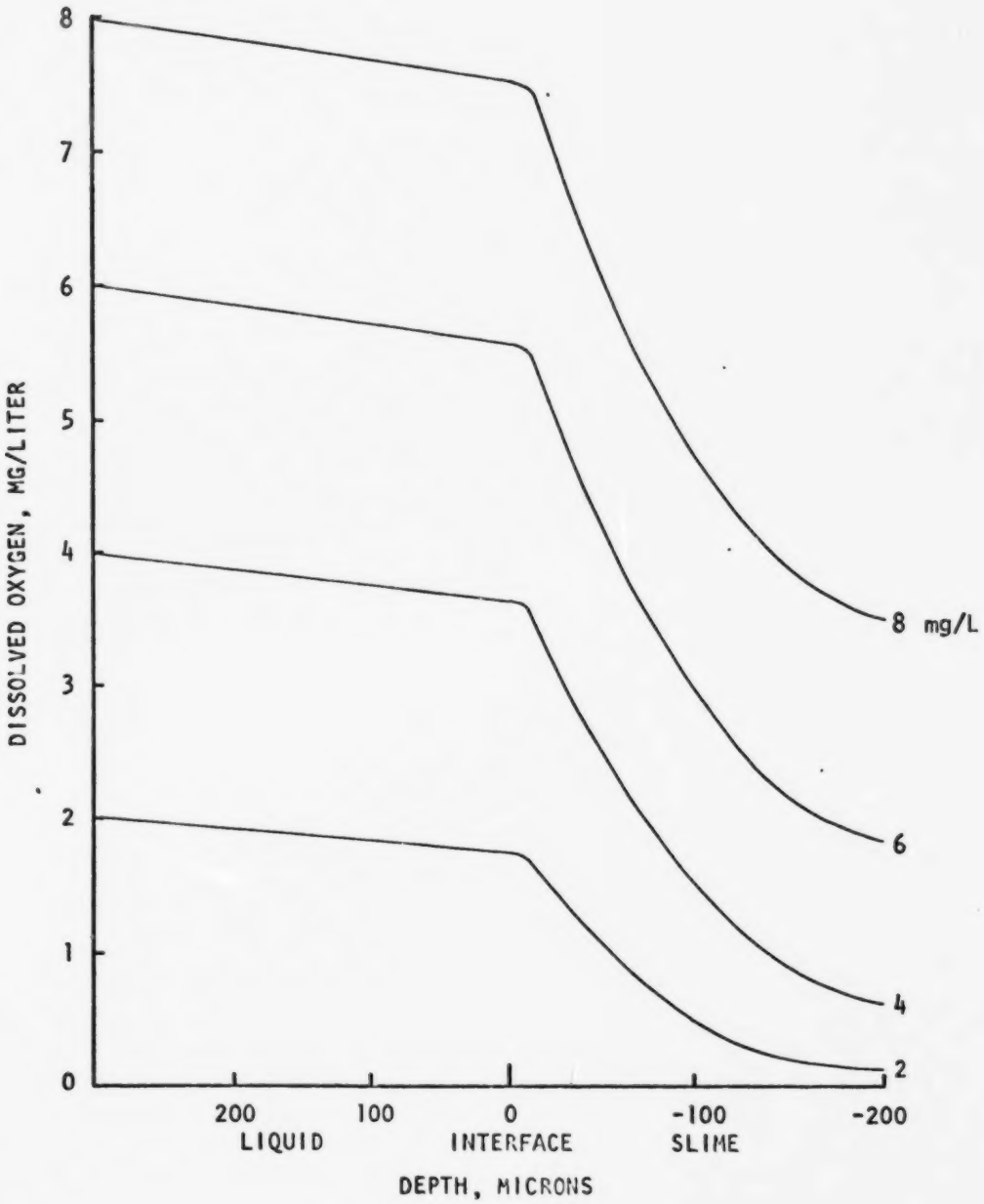

FIGURE 6

Effect of Inlet Oxygen Concentration on Dissolved Oxygen Profile

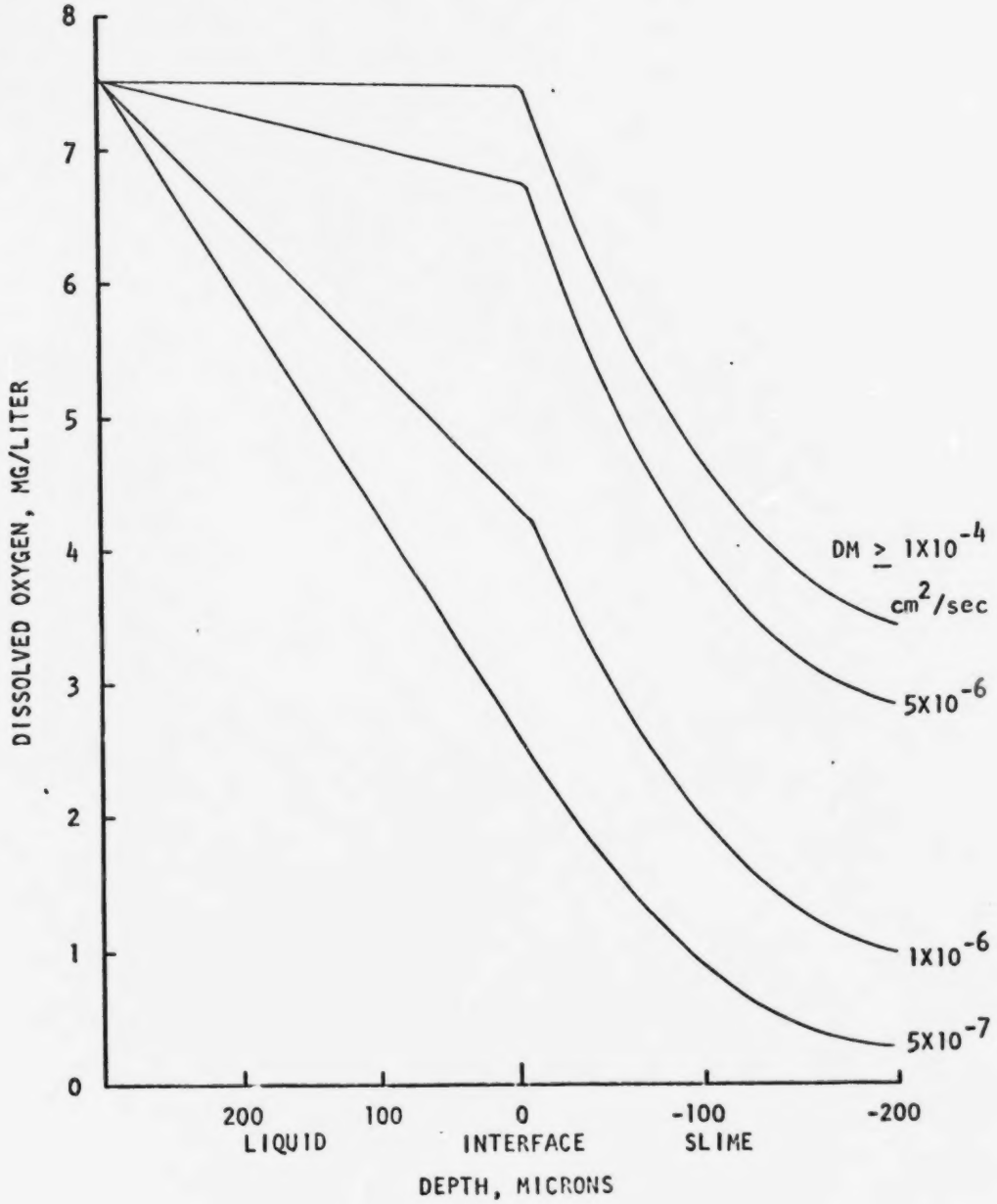

FIGURE 7

Effect of Oxygen Diffusivity in the Liquid

on Dissolved Oxygen Profile

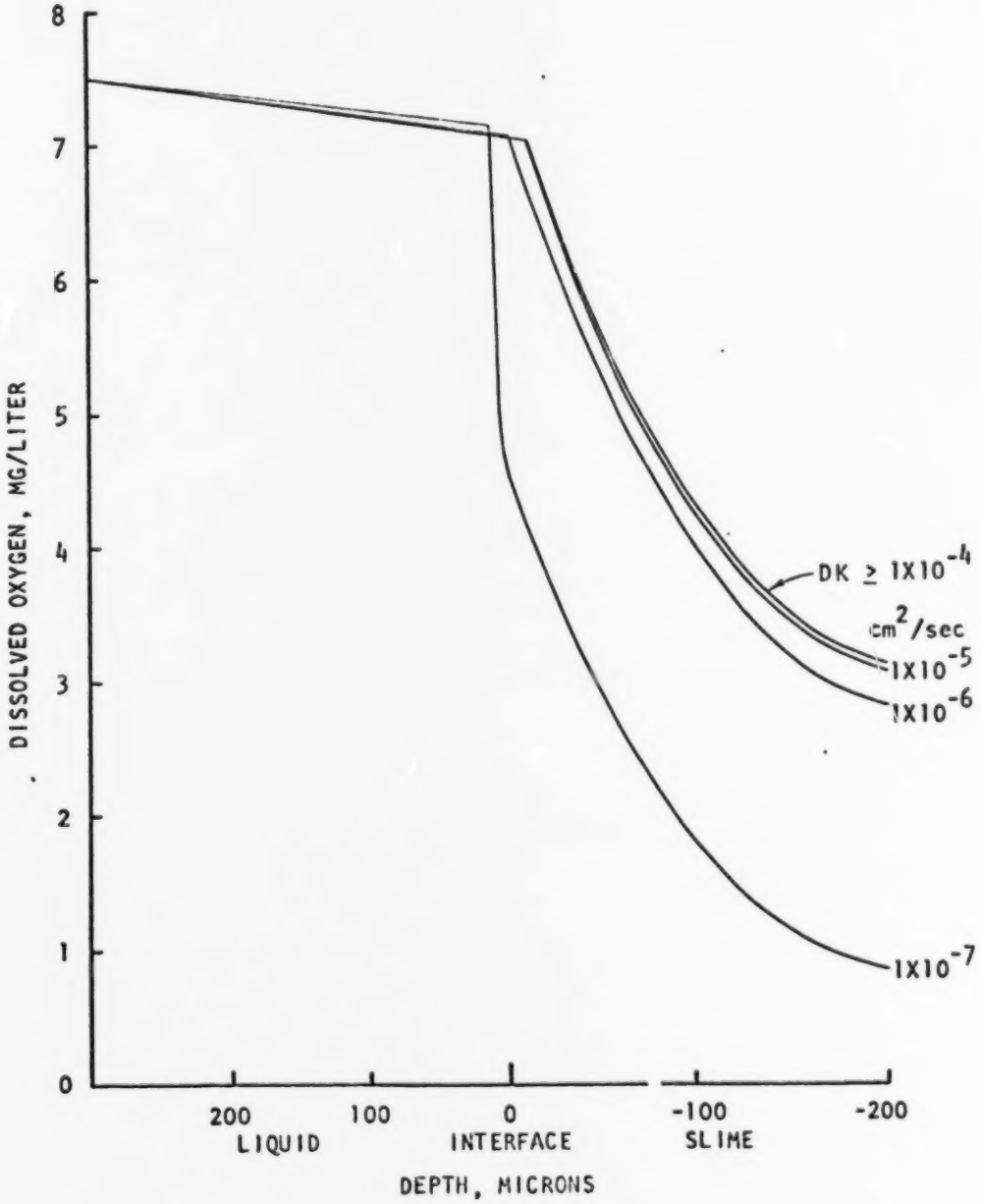

FIGURE 8

Effect of Interface Resistance on Dissolved Oxygen Profile

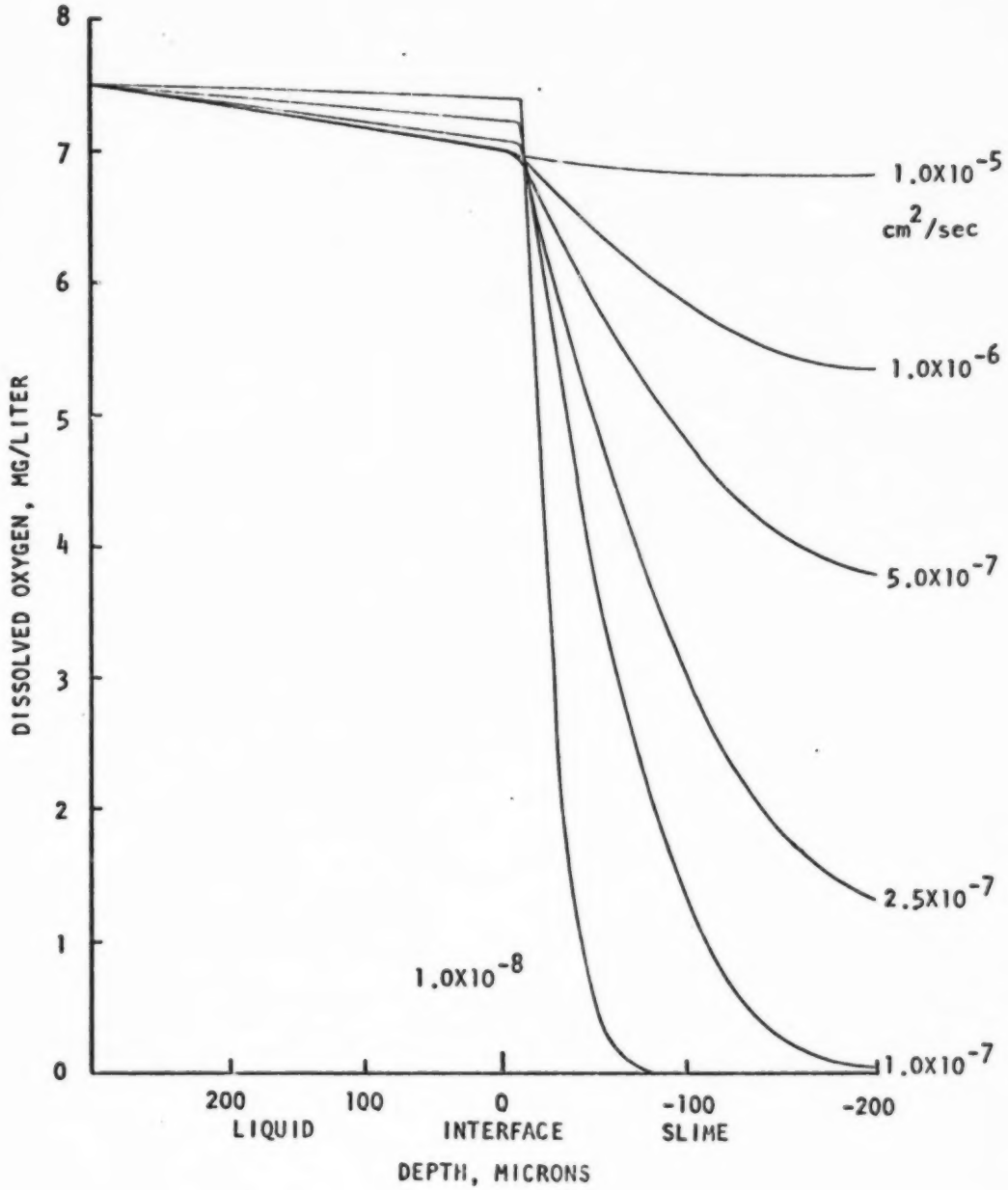

FIGURE 9

Effect of Oxygen Diffusivity in the Slime

on Dissolved Oxygen Profile

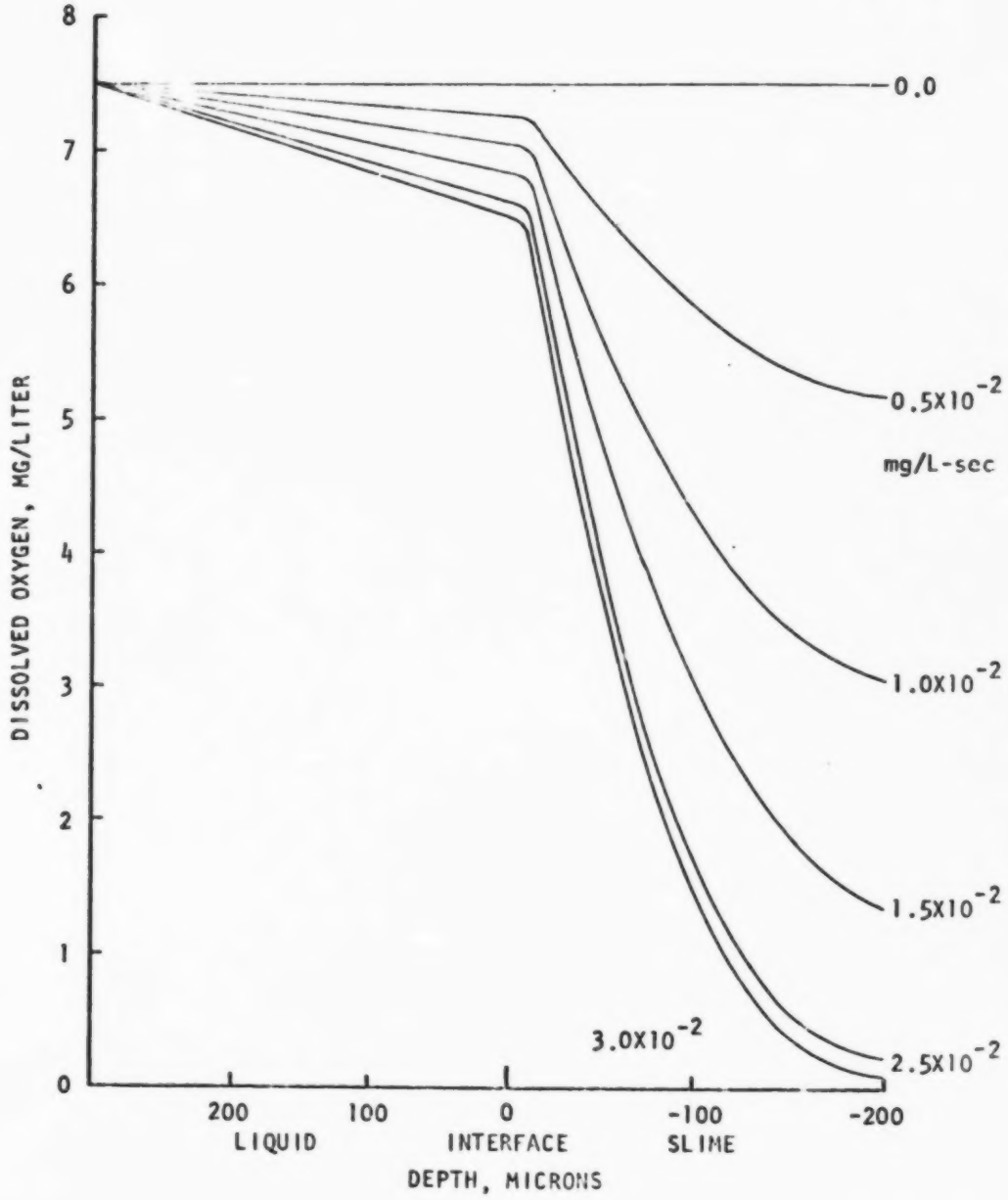

FIGURE 10

Effect of Value Selected for Maximum Uptake Rate

on Dissolved Oxygen Profile

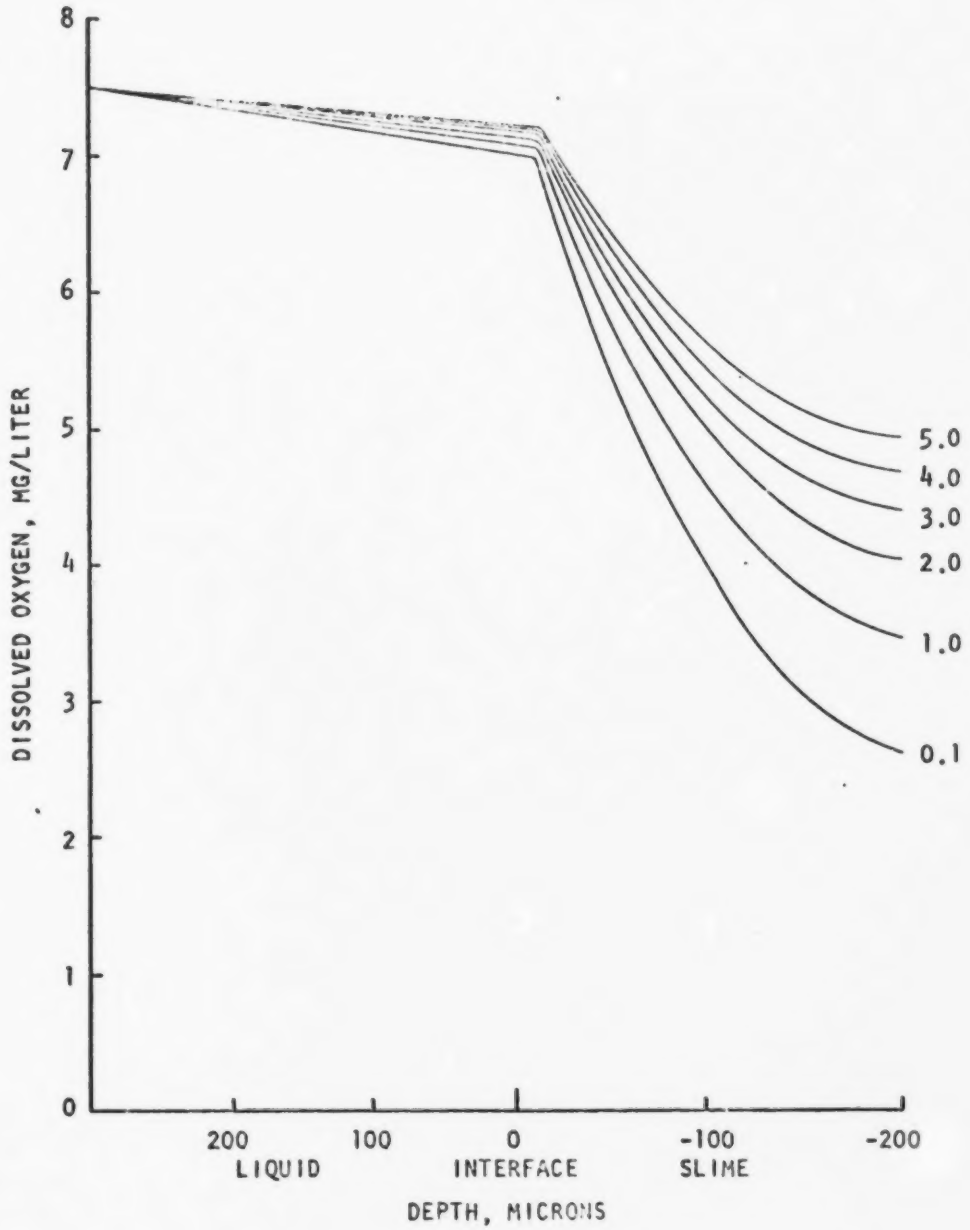

FIGURE 11

Effect of Constant in Denominator of Monod Equation

on Dissolved Oxygen Profile

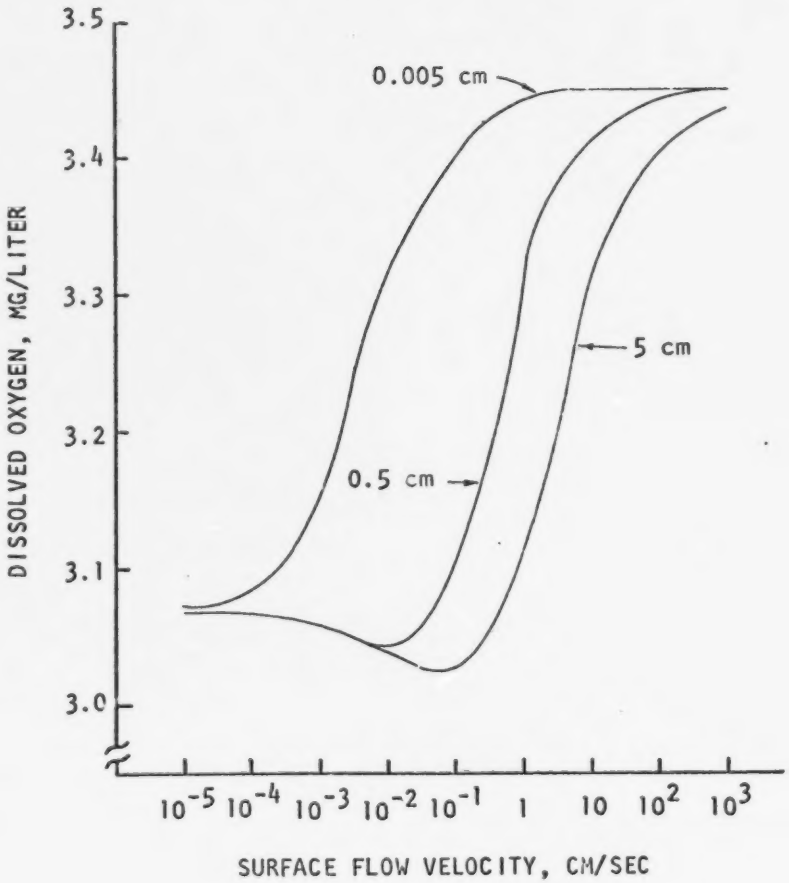

FIGURE 12

Effect of Flow Velocity of Medium on Dissolved Oxygen Concentration in the Deepest Slime Layer for Various Slime Thicknesses

upper layers. Thus layers close to the surface may be aerobic while organisms deep within the film encounter anaerobic conditions.

Changes in dissolved oxygen concentration of the feed stream did not cause a proportional change in the dissolved oxygen profile as might be expected (Figure 6). Comparing the profiles for each oxygen feed concentration, the portion of the profile in the liquid changed proportionally to changing feed stream concentration; however, profiles in the deeper layers of the slime seemed distorted at low concentrations when feed stream concentration was changed. This was caused by a nonlinear function relating oxygen consumption to oxygen concentration.

The effect of altering oxygen diffusivity of the liquid medium (Figure 7) was a drastic change in the profile, not only in the liquid but in the slime as well. As a resistance to oxygen diffusion increased, the oxygen profile dropped. However, increasing the diffusivity above 1×10^{-4} square centimeters per second. This results because a value greater than this would indicate a diffusivity greater than that in the liquid medium. However, below this value the interface would diffuse oxygen at a rate less than that of the liquid medium. Consider, for instance, the extreme case when the interfacial film coefficient is zero; then the profile because constant within the liquid and falls abruptly to zero at the interface.

Resistance to oxygen transfer in the slime was increased when the diffusivity was decreased (Figure 9). Since a small change in diffusivity greatly shifts the profile, the value of diffusivity is an important matter. Notice the decrease of slope for the profile in the liquid as the diffusivity of the slime increased. This was caused by the increased resistance to oxygen transfer in the slime from the decreased in slime diffusivity.

The maximum rate of oxygen consumption caused the profile to become less sensitive to uptake rate as the uptake rate increased (Figure 10). With this parameter a broader range of profiles may be generated than with any other parameter.

Changes in the constant G_k, of the Monod equation affected the profile over a limited range (Figure 11). As G_k was decreased, greater values of oxygen uptake were generated for a given concentration. This oxygen depletion made the profile steeper.

The effect of changing the surface liquid flow velocity did not change the rate of oxygen transfer appreciably (Figure 12). In the range from 0.0001 to 100 centimeters per second, velocity changes caused major changes in the amounts of oxygen in the lower layers; however, velocities outside this range had little affect. These oxygen concentration changes, although, were only a few parts of one milligram per liter.

Comparison of Model to Microprobe Data

Data from microprobe studies (1) were compared to computer predictions. In studies with living slimes, oxygen utilization rates within the slime were calculated from quite linear changes in oxygen concentration for roughly a twenty-second period during the early phases of collapse of the oxygen field. The oxygen left the slime because of a shift to nitrogen-sparged medium. From these studies a value of 2.5×10^{-2} milligram per

liter per second was picked as a reasonable estimate of oxygen uptake rate for elements not too deep in the slime. Figure 3 shows the dissolved oxygen profile for the data taken from microprobe studies. Using the computer to rest for a fit to this profile, adjustments were made in the rate of oxygen utilization. From repeated trials, a value of 2.57×10^{-2} milligrams per liter second was found to five a profile which fitted the experimental profile. In the lowest layers the computer profile stabilized at a value of 0.25 milligrams per liter of dissolved oxygen at a distance of four centimeters.

SECTION VI

SUPPORTING STUDIES

In recent years there has been an effort to develop a more theoretical approach to the study of the trickling filter in hopes of getting a better insight into the controlling factors which define its ultimate capacity and the limitations. The trickling filter is a contacting device which converts organic pollutants into cell mass. The microorganisms absorb the waste organic matter and metabolise it through respiration involving oxygen. As the cell mass becomes too great for attachment to the supporting medium, large portions slough off and flow out with the effluent. Accumulations of cell mass are recovered by sedimentation in a subsequent step. The sludges from sedimentation are disposed by several processes; major alternatives are sludge digestion, filtration, or incineration. Physically the trickling filter is a collection of supporting material to which films of microorganisms adheres. Waste water is applied by stationary or rotating distributors and percolates down through the packing to be discharged at the bottom. The inert packing acts as a support for biological growth; the porous structure and void spaces allow liquid and air to flow concurrently and simultaneously.

Maier, Behn, and Gates attempted to relate a simple laboratory element to the mechanism of sewage purification using trickling filters (2). They allowed liquid to flow over an inclined flat surface in which slime organisms were imbedded in a layer of window screens. The total thickness was kept constant by periodically scraping the upper surface of the screens. Data were taken for input and effluent concentrations of organic matter. A mathematical model was developed to relate transfer of nutrient and utilization of the organic substrate by considering a tiny element of volume. The assumption was made that there was no production or use of nutrients by an element in the liquid.

When the rate of use of nutrient at the slime-liquid interface is fast compared to the rate of transfer of nutirient from the bulk of the liquid film to the interface, nutrient is used as soon as it reached the interface.

Their equations were evaluated and the solutions compared with experimental results. Liquid feed rate was found to have a marked effect on the rate of glucose removal at feed rates below 20 milliliters per minute; but at high feed rates the rate of glucose removal became independent of feed rate. The rate of glucose removal was increased either by raising the temperature or by increasing the glucose concentration in the feed system. It appears that waste removal from water is limited by the rate of diffusion of substrate and oxygen from the liquid film to the slime layer.

Kornegay and Andrews (3) investigated the kinetics of fixed-film biological reactors. A completely mixed annular reactor was selected to permit the greatest degree of control and to facilitate measurements. A substrate consisting of glucose and minerals was fed to a series of annular reactors in which attached microorganisms greatly predominated over those in the completely mixed liquid phase. The annular reactor consisted of an enclosed outer vessel which contained volume, and increase the available

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surface for biological films. A slide fitted into the side wall could be removed for determination for thickness and density of the biological film. Their model was based on the following assumptions:

1) Complete mixing of the liquid phase was achieved.

2) Substrate utilization occurred entirely from metabolism of attached films; use from unattached organisms was small and could be neglected.

3) Rate of removal of substrate from the liquid phase was described by a saturation function.

Utilization of both dissolved oxygen and substrate by the biological film showed no further increase after the film attained a thickness of 70 microns. Examination of the theoretical curve depicting substrate removal by the liquid and observed results for the completely mixed fixed-film reactor indicated three facts. First, the rate of substrate removal from the liquid approached a constant value as the substrate concentration was increased. Second, the effluent concentration was dependent on the influent concentration. Third, the agreement found between the predicted and observed results supported the validity of the mathematical model. The relationship between the rate of substrate removal and concentration was linear at low concentrations but approached a saturation value at higher concentrations.

Atkinson, Swilley, Busch, and Williams (4) have derived a mathematical model for a respiring microbiological film. Two possible geometric models of the interface between the microorganisms and the liquid phase were considered. One referred to as the pseudo-homogeneous model, consisted of a branching matrix of microorganisms through which the liquid phase passed. The other, the hetrogenous model, consisted of organisms having as their exterior a smooth surface over which the liquid phase flowed.

A mixed population of microorganisms can utilize, in the absence of oxygen, a number of oxidants; nitrate is included among these oxidants. A real system using nitrate as the oxidant in the absence of oxygen was devised. The apparatus consisted of an inclined plate covered with a uniform layer of organisms. The surface was enclosed in a Perspex box containing a nitrogen atmosphere and it was fed with concentrated liquid medium continuously. The effect of a change in the angle of support surface on fraction of glucose removed was noted. Comparison between experimental values and predicted values showed that mathematical models derived on a heterogeneous basis are applicable to the biological film reactor.

The model of a heterogeneous biological film has been taken to a higher level of sophistication by Atkinson, Daoud, and Williams (5) by developing a predictive equation for the performance of the biological film reactor.

Sanders (6) suggests that natural slime communities consists of heterotrophs which are dependent upon the oxidation of organic substances for their sources of energy. Thus the respiratory activities of these microorganisms constitute the primary demand for oxygen in a typical aquatic

environment. He discusses three distinct phases in the growth and development of a slime community. However, Sanders emphasized the steady state phase of this development. This covers the growth of the organisms from the time the film has reached the limiting thickness for the diffusion of oxygen until the slime film sloughs off the surface. Once this dynamic balance results in a constant rate of oxygen utilization and a constant rate of removal of nutrients from the supernatant solution. In addition, Sanders shows that as the slime thickness increases the availability of nutrients and oxygen to the lower layers becomes limited.

The relation between oxygen concentration and respiration in Candida utilis growing on acetate was determined by Johnson (7). When grown in a continuous fermentor at various dissolved oxygen concentrations with time allowed for full adaptation to each oxygen concentration, the relationship between oxygen concentration and growth rate simulated Michaelis-Menten behavior.

Microprobe electrodes capable of measuring a variety of specific species such as Na^+, K^+, H^+ and Ca^{++} have been developed. Quite recently glass capillary microelectrodes have been developed for measuring dissolved oxygen concentrations. Typical electrodes can be drawn to tips in the range of one to two microns and can be positioned to an accuracy of $\pm$ 2 microns. Microelectrodes of this type permit a completely new approach to the study of mass transfer to oxygen.

One case that has been studied was an established film bathed continuously in flowing nutrient solution with various levels of dissolved oxygen. Using a microprobe electrode, Whalen, Bungay, and Sanders (8) have measured dissolved oxygen concentrations above the surface and within a bacterial slime mass. With dilute medium the oxygen profile became level at high concentrations within the film, indicating substrate-limited respiration. More concentrated medium caused the profile to fall to oxygen concentrations characteristics of oxygen-limited respiration.

For changes in nutrient or oxygen concentrations in the feed stream, steady state and transient measurements of oxygen concentration in a bacterial slime mass were used by Bungay, Whalen, and Sanders (1) to determine mass transfer coefficients.

Factors affecting the biological oxidation of sewage in percolating filters were investigated by Tomlinson and Snaddon (9) using an apparatus in which a thin film of sewage flowed over a layer of microorganisms which grew on the inner surface of an inclined rotating tube. The mathematical formula used in calculating the coefficient of diffusion of oxygen in slices of animal tissue, lead to two values for the diffusion of oxygen in microbial films. Under steady conditions if R is the rate of oxygen consumption by the organisms and this rate is independent of concentration of dissolved oxygen above a limiting value C_1, then

$$d_c^2 = \frac{D\,(C_0 - C_1)}{R} \qquad (11)$$

where C_0 is the saturation concentration of oxygen, D is the diffusion coefficient and d_c is the depth at which respiration ceases. The critical depth, d_c, was found experimentally by allowing a bacterial film to grow over a preformed layer of nitrifying bacteria and by using the extinction of ammonia oxidation as an indication of the virtual absence of dissolved oxygen. One value of coefficient of oxygen diffusion was calculated from the rates at which microorganisms produced ethanol and carbon dioxide when air was passed through the tube; the second was calculated from direct measurement of the rate of uptake of oxygen and the rate of production of carbon dioxide. With films containing mostly bacteria and with sewage as the substrate, the diffusion coefficient was 1.5×10^{-5} square centimeters per second. With film grown on a glucose medium and containing a high proportion of fungal mycelium the diffusion coefficient was 22×10^{-5} square centimeters per second. There is evidence that these high values result from the greater capacity of the fungal cells for transporting metabolites along the hyphae by protoplasmic streaming.

The mechanism of oxygen transfer through a pure culture floc of Zoogloea ramigera I-16M has been considered by Mueller, Boyle, and Lightfoot (10). Equations estimating the oxygen transfer across the liquid film surrounding the floc particle were written using mass transfer correlations for flow past spheres.

SECTION VII

DISCUSSION

Although this was a modest project with relatively very small expenses, the main objectives were accomplished. The computer simulation accounts for most of the variables of interest for a microbial slime bathed in flowing nutrient, and the calculated trends compare well with the behavior of real slimes. Quite good quantitative agreement was obtained with the limited data presently available for dissolved oxygen concentrations in slime films.

The elements of novelty in the model are (1) recognition of a tridiagonal matrix of coefficients which needed to be inverted only once per run and could be used for all the rest of the slices, and (2) use of a recursion routine for the non-linear relationship between utilization rate and oxygen concentration thus permitting the use of linear algebra, and (3) data display in the form of graphs prepared by the line printer or the CALCOMP plotter.

Almost any degree of accuracy can be obtained with the model by the selection of the number of horizontal slices in the medium and in the slime. However, the general assumptions for deriving the model do not justify the use of many slices to gain high accuracy. The model was intended as an aid to laboratory investigations of respiration in slimes, thus the main use should be to identify trends and leads to be explored in the laboratory.

While it is difficult to place a dollar value on a computer model, this model is intended to substitute for experiments with living systems. By checking the computer simulations with occasional laboratory data, it should be possible to advance much more rapidly toward greater understanding and thus improved performance of microbial slimes systems. The dollar saving in labor costs, laboratory runs, analytical determination, and the like can be considerable.

SECTION VIII

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The project officer, Dr. Walter M. Sanders, kept in close contact with the research providing advice and encouragement.

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SECTION IX

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SECTION X
SYMBOLS

$\overline{A}$	Tridiagonal coefficient matrix
$\overline{A}^{-1}$	inverse of matrix A
$\overline{B}$	vector of initial conditions and liquid flow velocities
C	oxygen concentration per unit volume
C_0	saturation concentration of oxygen
$\overline{C}$	solution of the system of equations
G_k	constant in Monod equation
$\overline{I}$	identity matrix
N,M	exponent
R	rate of oxygen consumption per unit volume
$\overline{R}$	oxygen utilization rate vector
R_{max}	maximum rate of oxygen consumption per unit volume
t	time
u	point liquid flow velocity
u_{max}	liquid film surface velocity
x,y,z	space coordinates
x*	liquid depth relative to liquid surface
δ	liquid film thickness
$\Delta x, \Delta y$	infinitesimal quantities of length
ΔXm	thickness of incremental layer in liquid
ΔXs	thickness of incremental layer in slime
ΔY	width of slice increment

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SECTION XI

PATENTS AND PUBLICATIONS

A highly detailed description of the computer model is available in the M. S. thesis of D. M. Harold, Jr., "A Computer Simulation of Oxygen Profiles in Microbial Systems," Clemson University (1970).

No Patents will arise from this project.

This computer model has been published. H. R. Bungay, III and D. M. Harold, "Simulation of Oxygen Transfer in Microbial Slimes" Biotechnol. and Bioengr. 13 569 (1971).

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